Pharmacology Biochemistry & Behavior, Vol. 21, pp. 301–311, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Dopamine Autoreceptor Mediation of the Effects of Apomorphine on Serotonin Neurons

EMINY H. Y. LEE AND MARK A. GEYER[1]

Departments of Neurosciences and Psychiatry, School of Medicine
University of California, San Diego, La Jolla, CA 92093

Received 3 November 1983

LEE, E. H. Y. AND M. A. GEYER. *Dopamine autoreceptor mediation of the effects of apomorphine on serotonin neurons*. PHARMACOL BIOCHEM BEHAV **21**(2)301–311, 1984.—The effects of direct apomorphine (APO) infusion to the dorsal raphe and the substantia nigra on serotonergic neurons were examined in male rats. The results showed that APO infusion to the dorsal raphe failed to produce a significant effect on serotonin (5-HT) neurons in the dorsal raphe or 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the corresponding projection site, the striatum. Conversely, direct APO infusion to the substantia nigra mimicked the effects of systemic APO, namely, elevated 5-HT fluorescence in the dorsal raphe and increased 5-HT and 5-HIAA concentrations in the striatum. Serotonin neurons in the median raphe and its projection site, the hippocampus, were unaffected. Furthermore, horseradish peroxidase injection to the dorsal raphe resulted in specific cell labelling in the substantia nigra and fiber labelling in the ventral tegmental area. Together with previous findings that the serotonergic actions of systemic APO were antagonized by haloperidol or intraventricular 6-hydroxydopamine pretreatment; and the selective dopamine (DA) autoreceptor agonist 3-3-hydroxyphenyl-N-n-propyl-piperidine mimicked the effects of APO on 5-HT neurons, these results suggest that the observed effects of APO on the mesostriatal serotonergic system are probably mediated through DA autoreceptors in the substantia nigra and possibly by a direct nigroraphe pathway.

Serotonin Dopamine Apomorphine Nigroraphe pathway Horseradish peroxidase Cytofluorimetry Microspectrofluorimetry High performance liquid chromatography Substantia nigra Dorsal raphe Striatum Neurotransmitter interactions

IN a series of experiments conducted previously, we demonstrated that the dopaminergic agonist apomorphine (APO) preferentially elevated serotonin (5-HT) fluorescence in the dorsal raphe and 5-HT and/or its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the corresponding terminal area, the striatum. Serotonin neurons in the median raphe nucleus or its terminal area, the hippocampus, were unaffected by APO [17]. Additionally, these effects were blocked by the dopaminergic antagonist haloperidol [17] or by intraventricular 6-hydroxydopamine (6-OHDA) administration [18]. Furthermore, the selective dopamine (DA) autoreceptor agonist 3-3-hydroxyphenyl-N-n-propyl-piperidine (3-PPP), given subcutaneously, mimicked the serotonergic actions of APO in both the cell body (dorsal raphe) and the terminal (striatum) regions of the mesostriatal serotonergic pathway without affecting 5-HT neurons in the median raphe or the hippocampus [19].

Recent anatomical, biochemical and electrophysiological studies suggest that the substantia nigra receives direct monosynaptic inhibitory inputs from the dorsal and median raphe nuclei and that these pathways use 5-HT as a neurotransmitter [1, 4, 6, 10, 11, 12, 23, 24, 25]. While many reports suggest the raphe to nigra projections, few studies have been done to investigate the reciprocal nigroraphe projection. One HRP study indicated that the dorsal raphe nucleus, particularly its rostral part, receives direct projections arising from the substantia nigra [25]. Similarly, small injections of tritiated leucine or proline confined to the pars compacta of the substantia nigra label fibers that are distributed in the ventral half of the central gray area including the dorsal raphe nucleus [3].

The present study was designed to more precisely localize the effects of APO on 5-HT neurons. In awake, unrestrained rats, APO was directly infused into the dorsal raphe or the substantia nigra prior to both cytofluorimetric measures of 5-HT in the raphe nuclei and biochemical estimations of 5-HT and 5-HIAA in the striatum. Locomotor activity was also measured in animals during the intranigral APO infusions. In separate animals, HRP was injected into the dorsal raphe area for a retrograde transport study. It was hypothesized that if DA autoreceptors in the substantia nigra mediate the effects of APO on 5-HT neurons and the nigroraphe projection does exist, then nigral infusions of APO should mimic the effects of systemic APO and HRP injection to the dorsal raphe should result in cell labelling in the substantia nigra.

[1]Requests for reprints should be addressed to Dr. Mark A. Geyer, Department of Psychiatry, T-004, UCSD, La Jolla, CA 92093.

GENERAL METHOD

Animals

The animals were 88 experimentally naive, male, Sprague-Dawley rats weighing 250–350 grams. Upon receipt from the supplier (Charles River Laboratories), the rats were housed in pairs in a temperature-regulated (25±2°C) animal room on a 12/12 hr light/dark cycle. Food and water were available throughout.

Drugs

Apomorphine hydrochloride (Merck Company, Rahway, NJ) and 1% wheat germ agglutinin conjugated horseradish peroxidase (Sigma) were used. Apomorphine was dissolved in 0.9% isotonic saline immediately before use. Due to rapid decomposition, a fresh APO solution was prepared every two hours. Doses refer to the salt form.

Apparatus

The microspectrofluorimeter is described in detail elsewhere [14]. Briefly, a 1000 watt Xenon lamp and grating monochromator (Schoeffel) provides 410 mm excitation light that is admitted to the specimen under computer control through an electronic shutter. A Leitz microscope and MPV system with substage phase-contrast red light and a Ploem epi-illuminator allow for alignment of the specimen in phase-contrast and the isolation of a 5 μm circular area of the cytoplasm adjacent to the nucleus for fluorescence measurement. Alternatively, the measurement aperture is aligned in the regions between cell bodies. This extraperikaryal measure has been found to respond to some drugs independently of changes in the intracellular measure [14]. A small grating monochromator is used to select the optimal emission wavelength (512 nm) for biogenic amines to be detected by photometer. After alignment of each cell, readings are taken automatically at one per second and stored on magnetic tape for subsequent analyses. Both model droplet analyses and *in vivo* studies with pargyline have shown that the fluorescence intensity remaining after 14 seconds of excitation is proportional to the concentration of catecholamines (CAs), while a fading measure—the difference in fluorescence intensities from the first to the fourteenth second of excitation—is the best predictor of 5-HT concentration and independent of CA levels.

Microscopy

Using red-light phase-contrast, the slides containing the regions of interest were selected by reference to predetermined landmarks. In these experiments, one rostrocaudal plane through the midbrain was selected for the dorsal and median raphe cell measures. For each region, at least six intracellular and six extraperikaryal readings were taken from two or three different sections. Four background readings were taken from non-fluorescent cell bodies in the lateral reticular formation from each section.

Tissue Preparation

At the appropriate time after injection, animals were sacrificed by decapitation and their brains were removed within 90 sec. To allow reliable comparisons among a large number of tissue samples, the formaldehyde histochemical method has been modified so that all samples within an experiment are batch-processed together from sacrifice until sectioning [14]. After dissection, brain samples were frozen in propane, freeze-dried over phosphorous pentoxide for four weeks at −60°C, treated with gaseous formaldehyde, embedded in paraffin, and sectioned at eight μm on a rotary microtome. Sections were mounted on slides in Entellan (Merck).

High Performance Liquid Chromatography (HPLC)

The electrochemical/chromatographic system used was the Bioanalytical Systems LC-17 equipped with a "μBondapack" c-18 reverse-phase column (3.9 by 30 cm, Waters Assoc.), an Altex pump and an LC-4 amperometric detector coupled to a TL-5 glassy carbon electrode (Bioanalytical Systems) and an Ag-AgCl reference electrode. Stainless steel tubing was used throughout. Output from this system was recorded with a Shimadzu Integrator and a Houston dual-pen recorder. Water for the solvent was deionized using a Millipore "Milli-Q" water purification system. Amines and their metabolites were estimated according to the methods of Mefford [20]. Slight modifications for 5-HT and 5-HIAA assays have been made, as described previously [17]. Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and norepinephrine (NE) were assayed as described elsewhere [18].

Surgery

Rats were anesthetized with Nembutal [40 mg/kg, intraperitoneally (IP)] and placed in a stereotaxic apparatus, with the tooth bar positioned at −2.4 mm. For dorsal raphe infusions, a 30 gauge needle was lowered 0.2 mm caudal to the central part of the dorsal raphe. The coordinates were: AP +0.3 mm; L +0.0 mm; DV +3.3 mm from ear bar. The infusion line was filled with drugs and connected to a 50 μl Hamilton syringe immediately before delivery. The infusion began two minutes after the needle had been lowered. Infusion was done by an infusion pump at the speed of 0.125 μl/min for 16 minutes. A total of 0.5 μg APO or 4 μg DA in two μl saline solution was delivered to the animals. Needles were slowly removed three minutes after the infusion was accomplished and animals were sacrificed by decapitation another five minutes later. Raphe brain slices were processed for fluorescence histochemistry. For substantia nigra infusions, 25 gauge guide cannulae were implanted bilaterally in the substantia nigra 2.0 mm lateral to the lambda, 1.6 mm rostral to lambda and 2.8 mm ventral to the surface of the skull. The cannulae were secured to the skull with stainless steel screws and dental cement.

Behavioral Pattern Monitor

The experimental chamber used for activity measures during intranigral APO infusions was a 30.5 cm by 61 cm box with walls 38 cm high and a stainless steel floor. The walls were black Plexiglas except for a 15.2 cm high stainless steel touchplate that detected wall rearings. A four by eight perpendicular array of photobeams 1.9 cm above the floor was used to localize the animal's floor position with 3.8 cm resolution. Each chamber was enclosed in an electrically shielded and ventilated wooden cabinet and was illuminated from above by a 15-W incandescent light. A microprocessor system checked the status of the beams and circuits in each chamber every 100 msec. If any change had occurred in that interval, a data reading was taken. The results were analysed as detailed elsewhere [13]. For the purposes of this study, only a simple measure of locomotion was used, namely crossovers from one 15.2 cm square sector to another.

Infusion Apparatus

In the substantia nigra infusion experiment, the infusion pressure was generated by a custom-made infusion pump. For each rat, two Hamilton 10 μl microsyringes were placed in the infusion pump, each connected to its own infusion line. The infusion lines consisted of PE-20 polyethylene tubing (Intramedic). Distal to the syringe each infusion line was attached to an infusion needle made of 30 gauge stainless steel tubing. When secured to the guide cannula, the needle protruded 2.8 mm below the tip of the cannula. The infusion lines ran down through a hole in the outer cabinet above the center of the chamber and were supported by a counterbalanced arm.

Histology

In the dorsal raphe infusion experiment, DA was infused to a separate group of animals at the same coordinates as used for APO infusion to approximate the distribution of locally infused APO by the bright DA fluorescence after formaldehyde reaction. For the substantia nigra infusion experiment, after animals were decapitated, the two mm brain slices containing the substantia nigra were frozen-sectioned in a cryostat. Eight-micron thick sections were examined continuously through the substantia nigra. Under the microscope, localizations of the needle track and needle tip in the brain were recorded for each animal in writing. In one control animal, methylene blue dye (3 mg/ml) was infused immediately before sacrifice for histological verification of cannula placement and spread of the infusate and for photography. Sixteen-micron thick sections also taken at 50 μm intervals through the substantia nigra were mounted on slides, some were stained with toluidine blue. The needle track and the distribution of dye was recorded photographically.

Statistics

In cytofluorimetric studies, separate analyses of variance (ANOVAs) were done for both intracellular and extraperikaryal measures from each brain region examined. The appropriate values from each animal were averaged after subtraction of the corresponding blank value so that each animal contributed only one value to each ANOVA. Specific comparisons between each treatment group and a common control group were made with Dunnett's t-test [29]. Similar analyses of variance and Dunnett's t-tests were also used to analyze the biochemical and behavioral data.

EXPERIMENT 1

In order to determine whether DA receptors in the dorsal raphe are responsible for the effects of systemic APO on the serotonergic system, APO was directly infused into the dorsal raphe in one group of animals (N=7). Dopamine was similarly infused to a separate group of animals (N=5). The purposes of DA infusion to the dorsal raphe were, first, to mimic the pharmacological action of APO on the serotonergic system, second, to approximate the distribution of locally infused APO by the bright DA fluorescence after formaldehyde reaction. Animals in the control group (N=7) received the same amount of vehicle infusion.

Results

The effects of APO and DA infusions on 5-HT fluorescence measures are illustrated in Fig. 1. As shown in this figure, neither APO nor DA infusion had a significant effect on any type of 5-HT fluorescence measure in the dorsal or median raphe. However, DA infusion to the dorsal raphe significantly increased the final fluorescence intensity, a measure of CAs, in this area, $F(2,14)=8.17$, $p<0.01$ (Fig. 2). Conversely, infusion of APO did not affect CA level in the extracellular space of the dorsal raphe. Neither drug affected CA fluorescence in the median raphe.

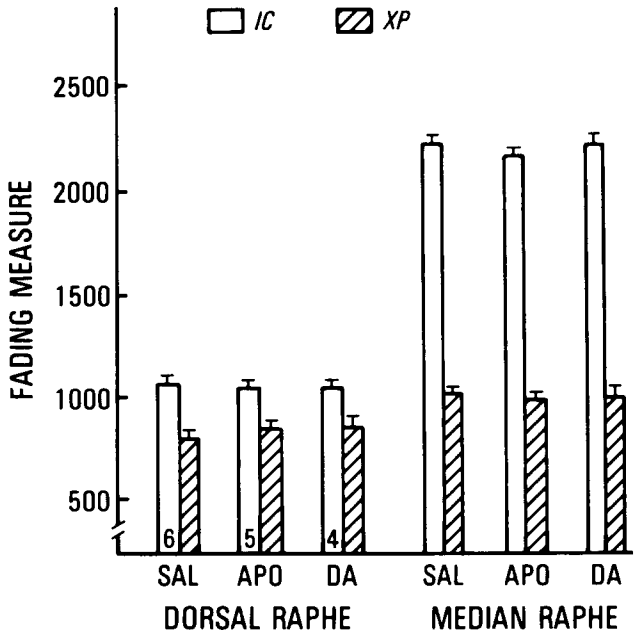


FIG. 1. Effects of APO (0.5 μg/2 μl) and DA (4 μg/2 μl) infusions to the dorsal raphe on 5-HT fluorescence fading measure in the dorsal and median raphe nuclei. The results show that in both regions neither drug had a significant effect on 5-HT fluorescence of the intracellular (open bars) or the extraperikaryal (hatched bars) fading measure. Each bar represents the mean±S.E.M. Group size is indicated by the number shown in each open bar.

Consistent with the histochemical results, Table 1 indicates that direct infusion of APO or DA to the dorsal raphe did not alter 5-HT or 5-HIAA concentrations in the striatum. Dopamine and DOPAC levels in the same area were unaffected by APO infusion; however, direct DA infusion to the dorsal raphe significantly elevated both DA and DOPAC concentrations in the striatum, $tD(3,15)=3.92$, $p<0.01$; $tD(3,18)=2.62$, $p<0.05$ (Table 2), without changing the rate of DA turnover.

EXPERIMENT 2

In order to test the hypothesis of DA autoreceptor mediation of the serotonergic actions of systemic APO, APO was directly infused to the substantia nigra in one group of animals (N=9). The control animals (N=9) received similar infusions of saline.

Procedure

Animals were brought into the animal behavior laboratory at least an hour before the start of the experimental session. At the start of the session the infusion needles were inserted into the rat's guide cannulae and secured. Each animal was placed in a holding cage and the infusion pump was turned

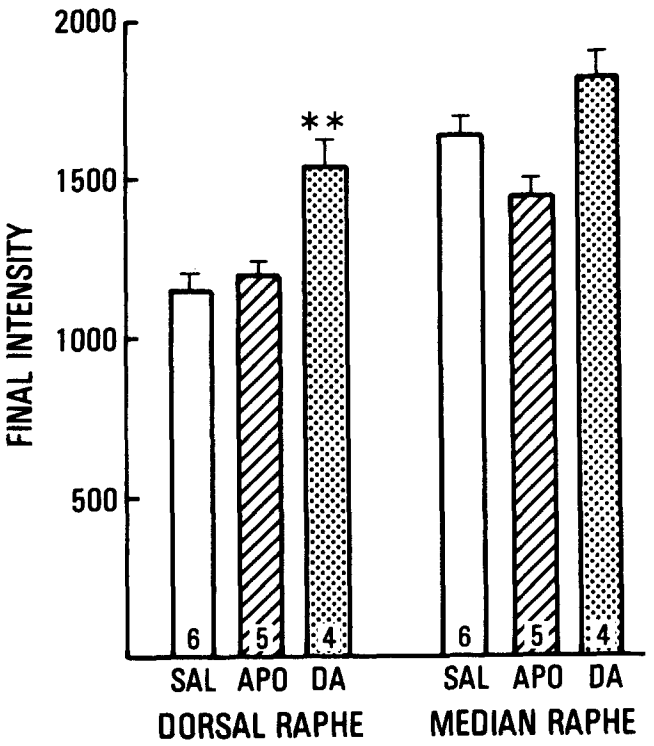


FIG. 2. Effects of APO (0.5 μg/2 μl) and DA (4 μg/2 μl) infusions to the dorsal raphe on final fluorescence intensity, CA fluorescence measure, in the extraperikaryal space of the dorsal and median raphe nuclei. Infusion of DA selectively increased CA fluorescence in the dorsal raphe nucleus without affecting CA in the median raphe nucleus. Infusion of APO did not have an effect on CA fluorescence in either region. Each bar represents the mean±S.E.M. Group size is indicated by the number shown in the open bar or right below the patterned bars. $^{**}p<0.01$.

TABLE 1

EFFECTS OF APO AND DA INFUSIONS TO THE DORSAL RAPHE ON 5-HT AND 5-HIAA LEVELS IN THE STRIATUM

Treatment	N	5-HT	% of control	5-HIAA	% of control
SAL	9	1232 ± 23		1004 ± 40	
APO	9	1280 ± 46	104	932 ± 33	93
DA	5	1258 ± 45	102	979 ± 44	98

Data are expressed as ng/g tissue. Values are means ± SEM and are not corrected for recovery.

TABLE 2

EFFECTS OF APO AND DA INFUSIONS TO THE DORSAL RAPHE ON DA AND DOPAC LEVELS IN THE STRIATUM

Treatment	N	DA	% of control	DOPAC	% of control
SAL	7	5077 ± 96		263 ± 20	
APO	7	5274 ± 230	104	280 ± 16	106
DA	5	6184 ± 196†	122	335 ± 12*	127

Data are expressed as ng/g tissue.

*$p<0.05$, †$p<0.01$. Values are means ± SEM and are not corrected for recovery.

on. Five minutes later, the rat was placed in the chamber to begin the test. Each session lasted 30 min, throughout which the rat received a bilateral intranigral infusion at the rate of 0.025 μl/min and a total of 0.75 μg APO in 0.75 μl saline solution was infused to each substantia nigra.

Results

Figure 3 illustrates the effects of nigral APO infusion on serotonin (5-HT) fluorescence in the dorsal and median raphe nuclei. Consistent with results obtained from previous studies [17], intranigral APO augmented both the intracellular and the extracellular 5-HT fluorescence, $F(1,11)=18.16$, $p<0.01$; $F(1,11)=7.82$, $p<0.05$, in the dorsal raphe without affecting 5-HT cells in the median raphe. The CA level in the extraperikaryal space of the dorsal raphe was also significantly elevated by intranigral APO, as shown in Fig. 4, $F(1,11)=22.11$, $p<0.01$. The same manipulation did not alter any type of 5-HT fluorescence measure in the median raphe.

As summarized in Table 3, APO infusion to the substantia nigra significantly elevated both 5-HT and 5-HIAA levels in the striatum, $F(1,12)=5.30$, $p<0.05$; $F(1,12)=8.51$, $p<0.05$. Dopamine and DOPAC concentrations in the same region were also increased by intranigral APO as revealed in Table 4, $F(1,12)=11.92$, $p<0.01$; $F(1,12)=4.94$, $p<0.05$.

Behaviorally, locomotor activity was significantly decreased in animals with intranigral APO application, as indicated in Table 5, $F(1,14)=4.61$, $p<0.05$.

Histologically, the position of the needle tip in most animals examined was localized at the rostral part of the substantia nigra pars compacta (Fig. 5). In a few animals, it was placed in between the substantia nigra pars compacta and pars reticulata. One animal was excluded from the experiment because the needle was outside the nigra.

EXPERIMENT 3

To further investigate the proposed nigroraphe projection, HRP was injected into the dorsal raphe in four animals at the plane A160 to 350 of König and Klippel [16]. Specific cell labelling in the substantia nigra was examined. In another two animals, HRP was injected into the lateral reticular formation in the same rostrocaudal plane as the dorsal raphe.

Procedure

Animals received an injection of 50 nl of 1% wheat germ agglutinin conjugated horseradish peroxidase delivered through a 1.0 μl Hamilton syringe over a 25 minute period. After 24 hours survival, rats were anesthetized with Nembutal and perfused with a solution of 0.9% sodium chloride followed by a solution of 1.0% paraformaldehyde and 1.25%

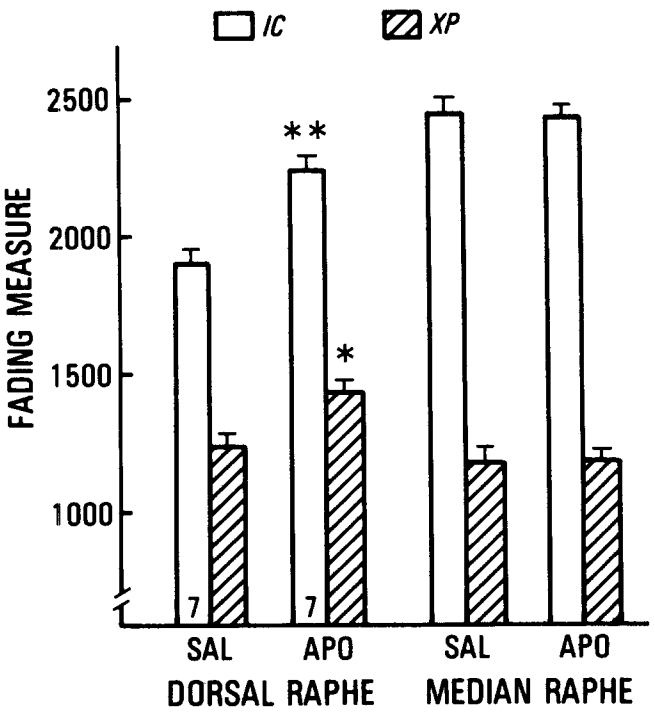


FIG. 3. Effects of APO infusion (0.75 μg/0.75 μl/per side) to the substantia nigra on 5-HT fading measure in the dorsal and median raphe nuclei. Infusion of APO selectively increased the intracellular (open bars) and the extraperikaryal (hatched bars) 5-HT fluorescence in the dorsal raphe nucleus without affecting 5-HT fluorescence in the median raphe nucleus. Each bar represents mean±S.E.M. of seven animals. *$p<0.05$ and **$p<0.01$.

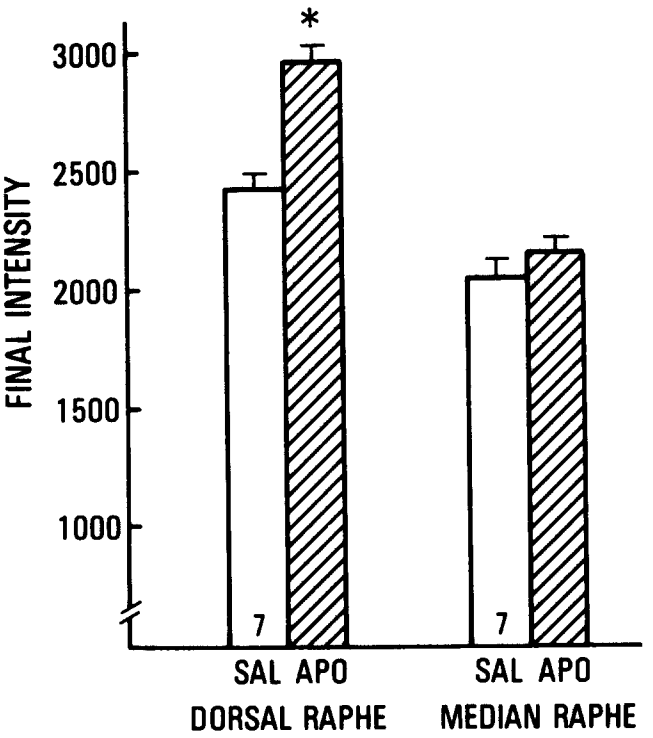


FIG. 4. Effects of APO (0.75 μg/0.75 μl/per side) infusion to the substantia nigra on final fluorescence intensity, CA fluorescence measure, in the extraperikaryal space of the dorsal and median raphe nuclei. Apomorphine infusion selectively elevated CA fluorescence in the dorsal raphe without affecting it in the median raphe nucleus. Each bar represents mean±S.E.M. Group size is indicated by the number shown in each open bar or right below the hatched bar. *$p<0.05$.

TABLE 3

EFFECTS OF APO INFUSION TO THE SUBSTANTIA NIGRA ON 5-HT AND 5-HIAA LEVELS IN THE STRIATUM

Treatment	N	5-HT	% of control	5-HIAA	% of control
SAL	7	1241 ± 27		689 ± 23	
APO	7	1348 ± 38*	109	772 ± 17*	112

Data are expressed as ng/g tissue.

*$p<0.05$. Values are means ± SEM and are not corrected for recovery.

TABLE 4

EFFECTS OF APO INFUSION TO THE SUBSTANTIA NIGRA ON DA AND DOPAC LEVELS IN THE STRIATUM

Treatment	N	DA	% of control	DOPAC	% of control
SAL	7	4985 ± 132		376 ± 17	
APO	7	5822 ± 203†	117	422 ± 13*	112

Data are expressed as ng/g tissue.

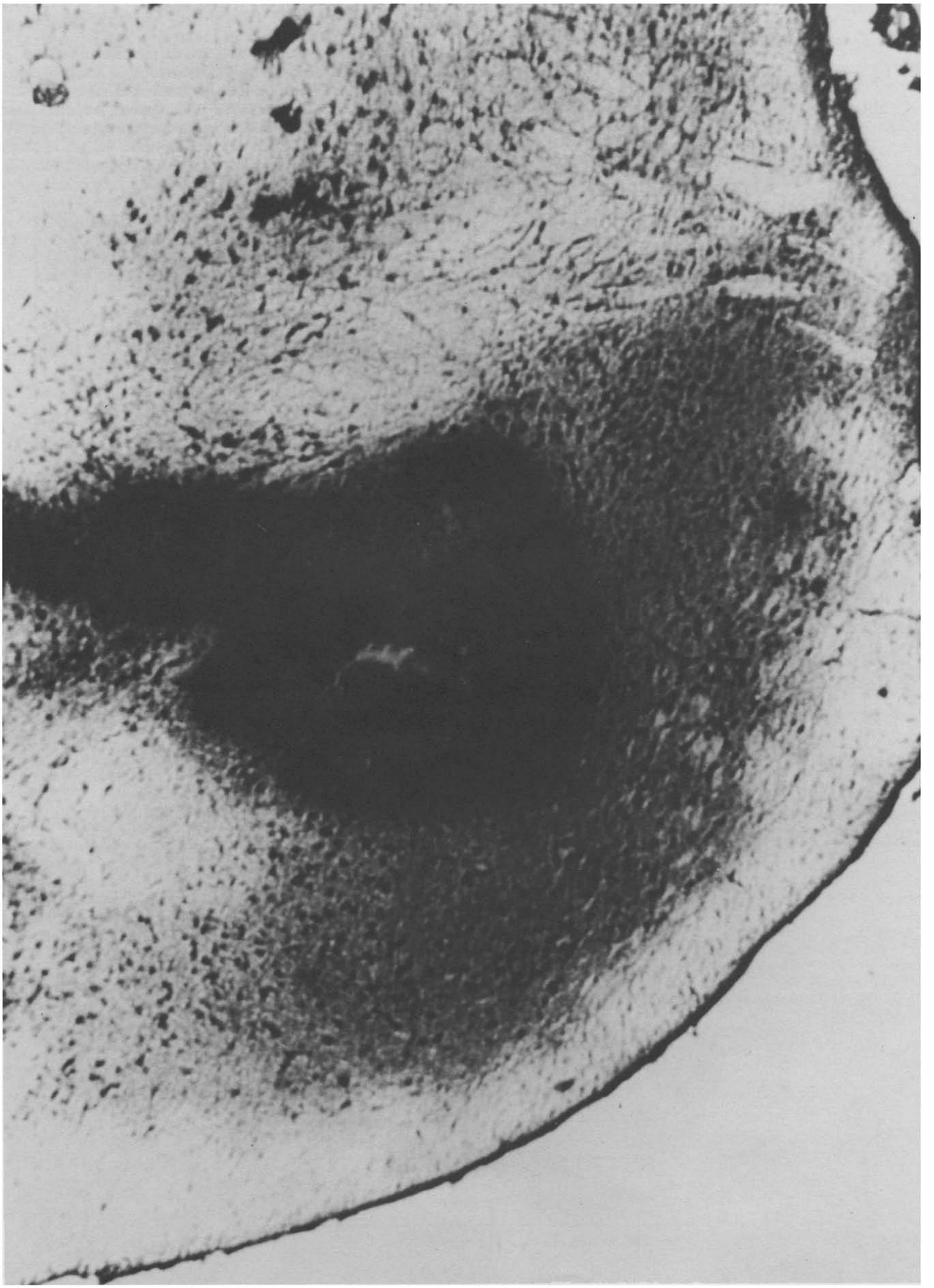
*$p<0.05$, †$p<0.01$. Values are means ± SEM and are not corrected for recovery.

TABLE 5

EFFECTS OF APO INFUSION TO THE SUBSTANTIA NIGRA ON LOCOMOTOR ACTIVITY

Treatment	N	Locomotor activity
SAL	8	1119 ± 96
APO	8	806 ± 110*

*$p<0.05$. Values are means ± SEM.



glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. This was followed by a solution of 10% sucrose in 0.1 M phosphate buffer for an additional 30 minutes. The brains were removed, allowed to sit overnight in 20% sucrose in phosphate buffer, and cut at 40 μm on a freezing microtome. The sections were collected in ice-cold 0.1 M phosphate buffer and processed for HRP histochemistry according to the procedure of Mesulam [21]. Reacted sections were then rinsed with distilled water, mounted on gelatin-coated slides, and allowed to dry 24 hours. Sections were then counterstained with Richardson's stain or 0.1% neutral red and 0.1% safronin-O and coverslipped.

A series of regularly spaced sections throughout the rostro-caudal extent of the substantia nigra and the dorsal raphe were analyzed with both bright and dark field optics for the number and distribution of retrogradely labelled cells.

Results

Four animals were successfully injected with wheat germ agglutinated HRP along slightly different rostro-caudal placements within the dorsal raphe area (Fig. 6A). A few retrogradely labelled cells could be seen within the ipsilateral dorsomedial portion of the substantia nigra parts compacta (Fig. 6B). The number of cells in the substantia nigra varied slightly depending upon the placement of the injection site in the dorsal raphe. In general, more retrogradely labelled cells were seen after the more rostral than the more caudal dorsal raphe injections. However, no specific cell labelling in the substantia nigra was observed in controls animals (Fig. 6C). In addition, the retrogradely labelled fibers coursed through the lateral part of the interpeduncular nucleus and the ventral tegmental area before entering the dorsal raphe (Fig. 7A) and this labelling was also not observed in control animals (Fig. 7B, 7C). However, from the number of cells labelled in the substantia nigra, it seems that the proposed nigroraphe pathway is a light projection.

EXPERIMENT 4

We have previously demonstrated that APO elevated CA fluorescence in the dorsal raphe [17]. We have also demonstrated that intraventricular 6-OHDA, given together with pargyline and desimipramine, decreased the CA fluorescence measure in the dorsal raphe without altering brain norepinephrine concentration [18]. These results indicate that the elevated CA in the dorsal raphe by APO is probably attributable to the effect of the drug on the dopaminergic system. An alteration of raphe DA levels by APO would be consistent with the existence of a direct dopaminergic afferent to the dorsal raphe. The present experiment was designed to test this hypothesis.

Forty-five animals were randomly assigned to two groups. Group 1 (N=20) received a single (IP) injection of saline; Group 2 (N=25) received a single injection (IP) of 1.0 mg/kg APO. Animals were sacrificed by decapitation 30 minutes following saline or APO injections.

Procedure

The dorsal raphe area in each animal was taken by a raphe punch (1.6 mm in diameter) and was processed for biochemistry of DA according to the methods described earlier. However, because of the relatively small size of the dorsal raphe (3.5–4.0 mg), samples from five animals in the same group were pooled together to achieve accurate assays. Other procedural differences were that 75 instead of 100 μl of perchloric acid was added to the homogenates for the final centrifugation step and two nA/V instead of five nA/V was used for better detection. The NE peak also came out in the same assay procedure and NE concentration was estimated simultaneously.

Results

The results of this experiment are summarized in Table 6. As revealed in this table, 1.0 mg/kg APO significantly increased DA in the dorsal raphe area, $F(1,8)=6.12$, $p<0.05$. Norepinephrine also appeared to be elevated (20%) in the same area, but this difference was not significant.

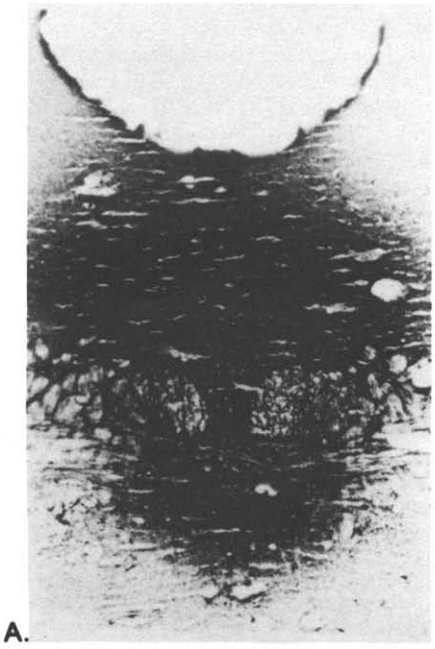
GENERAL DISCUSSION

In previous studies, systemic APO was found to consistently increase intracellular 5-HT fluorescence in the dorsal raphe and both 5-HT and 5-HIAA concentrations in the striatum [17]. Intraventricular 6-OHDA administration has been shown to block these effects [18] and 3-PPP mimics the effects of APO on 5-HT neurons [19]. In the present study, infusions of APO directly into the dorsal raphe had no effect on 5-HT neurons. Conversly, direct APO application to the substantia nigra produced the same effects as did systemic APO and 3-PPP. These results suggest that DA autoreceptors in the substantia nigra may be the anatomical locus mediating systemic APO's effects on the mesostriatal serotonergic pathway.

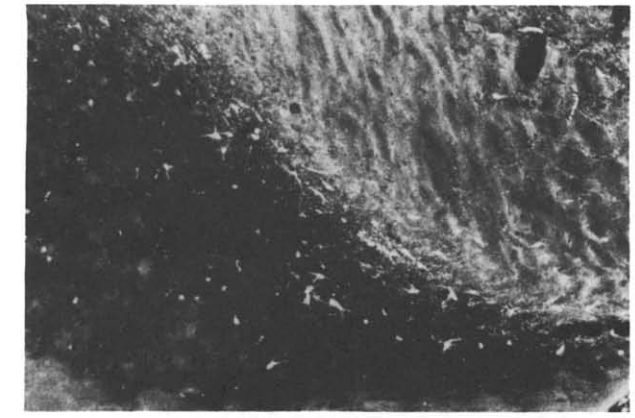
Electrical stimulation of cells in the substantia nigra has been shown to suppress dorsal raphe activity and this effect showed greater selectivity for the midline region than for more lateral cells [26,27]. Although the neurotransmitter which mediates this suppression was not identified, the same subgroup of raphe cells were also selectively affected by APO administration [17]. Together with the finding that the DA level in the dorsal raphe was significantly augmented by APO (see Table 6), these results suggest that DA is the neurotransmitter mediating these effects. Recently, several anatomical reports have also indicated a nigroraphe projection innervating the dorsal raphe [3, 23, 25] and the cells projecting to the dorsal raphe originate from the mediodorsal part of the substantia nigra [23]. This latter result is consistent with our finding that HRP injection to the dorsal raphe selectively labelled cells in the dorsomedial part of the substantia nigra (see Fig. 6B) while similar HRP injection to the lateral reticular formation did not result in the same retrograde axonal transport (see Fig. 7B). In the present HRP study, labelled fibers also passed by the interpeduncular nucleus and coursed through the ventral tegmental area before entering the dorsal raphe, indicating that this afferent projection to the dorsal raphe may be oriented somewhat medially in the midbrain. Little is known about the anatomical relationship between the substantia nigra and the median

FACING PAGE

FIG. 5. Localization of infused dye in the substantia nigra. A 16 μm thick section through the substantia nigra at approximately A660–850 of König and Klippel [16] is shown. The substantia nigra was infused bilaterally with methylene blue dye (3 mg/ml) at a rate of 0.025 μl/min for 30 minutes before sacrifice. The infusion site is localized mostly in the dorsomedial part of the substantia nigra pars compacta and partially in the substantia nigra pars reticulata.



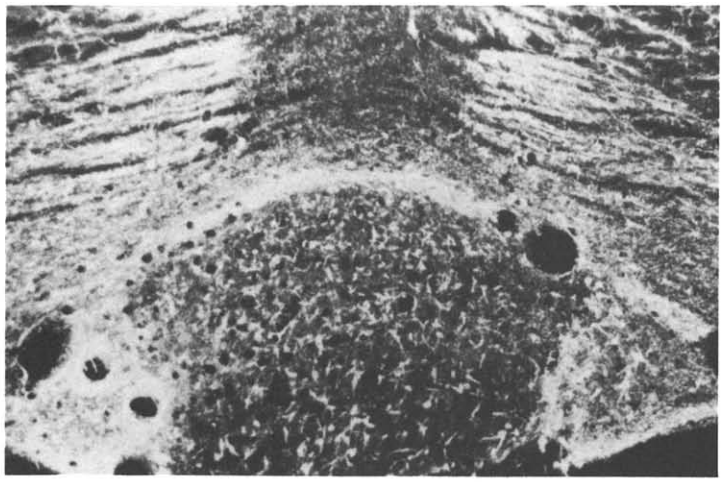
A.



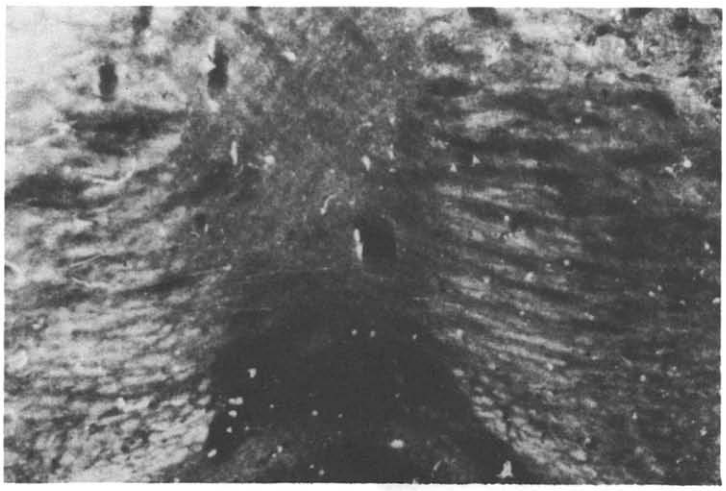
B.

C.

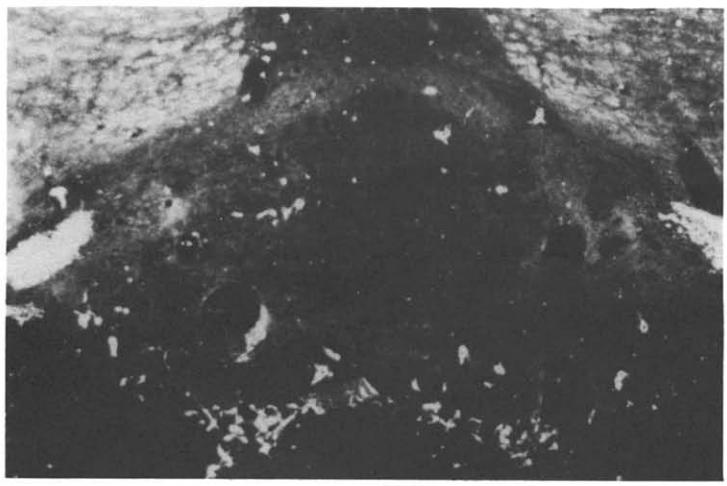
FIG. 6. (A) Photographic demonstration of HRP injection to the dorsal raphe nucleus in the midbrain. (B) Localization of HRP-labelled cells in the dorsomedial part of the substantia nigra following injection of HRP to the dorsal raphe nucleus. (C) No specific labelling of cells was observed in the substantia nigra following injection of HRP to the lateral reticular formation area (control group).



A.



B.



C.

FIG. 7. (A) Localizations of HRP-labelled fibers in the ventral tegmental area and HRP-labelled cells in the interpeduncular nucleus following HRP injection to the dorsal raphe nucleus. (B) Absence of the specific fiber labelling in the ventral tegmental area and (C) cell labelling in the interpeduncular nucleus following HRP injection to the lateral reticular formation.

TABLE 6
EFFECTS OF APO ON DA AND NE LEVELS IN THE DORSAL RAPHE

Treatment	N	DA	% of control	NE	% of control
SAL	5	40 ± 2		137 ± 8	
APO	5	53 ± 5*	133	165 ± 30	120

Data are expressed as ng/g tissue.
*$p<0.05$. Values are means ± SEM and are not corrected for recovery.

raphe except that Beckstead *et al.* reported some cell labelling in the anterior region of the median raphe after injection of tritated leucine and proline to the pars compacta of the substantia nigra [3].

Behaviorally, infusion of APO to the substantia nigra produced a significant decrease of locomotor activity in rats. This result is consistent with the notion that autoreceptor stimulation in the substantia nigra mediates behavioral sedation in animals [8, 9, 15].

Systemic APO administration has been shown to decrease DA synthesis and utilization by the evidence that tyrosine hydroxylase activity as well as DOPAC level were reduced in the striatum [7,28], while the dopaminergic antagonist haloperidol produced the opposite effects. However, in the present study, intranigral APO manipulation elevated both DA and DOPAC levels in the striatum. This result is consistent with the previous finding that 3-PPP also elevated DOPAC concentration in this area [19], while it contrasts with the result of another study [15]. This discrepancy could possibly be due to the different doses used (5.0 mg/kg vs. 1.0 mg/kg) or to different experimental procedures applied in these two studies. The augmentation of striatal DA content induced by bilateral intranigral APO is consistent with the result obtained from systemic application of APO. This significant effect on striatal DA contrasts with the ineffectiveness of much higher doses of unilateral intranigral APO reported previously [30].

Systemic [5] as well as intranigral [22] applications of APO have been shown to decrease the rate of DA neuronal firing, and to reduce DA release in the striatum. Furthermore, this inhibition of DA cell firing was still present following kainic acid injection into the striatum [2]. Similarly, the observed effects of APO on the mesostriatal serotonergic system might be attributable to an inhibition of DA neuron activity by APO. However, although decreased DA neuron firing would readily explain the acceleration of 5-HT turnover, it would not predict the increase of 5-HT synthesis as reflected by the elevated 5-HT level in the dorsal raphe and the striatum. These results suggest that mechanisms other than DA receptor stimulation by APO may also be involved in APO's effects on the serotonergic system. For example, DA could also be taken up by 5-HT neurons and influence the biosynthetic pathway of 5-HT by inhibiting tryptophan hydroxylase activity (Knapp and Mandell, personal communication). Thus, APO's inhibition of DA neuron firing might reduce the amount of DA inhibiting 5-HT synthesis in both the dorsal raphe and the striatum. Further studies are required to clarify the mechanism of APO's effects on 5-HT biosynthesis.

While the above results seem to support a direct dopaminergic projection from the substantia nigra to the dorsal raphe, other anatomical loci and polysynaptic pathways may also contribute to the effects of APO on 5-HT neurons. For example, an interaction could occur in the striatum since this area contains significant amounts of both DA and 5-HT. The HRP study also suggests that the ventral tegmental area may be another anatomical locus responsible for the observed effects of APO. It is also possible that some polysynaptic pathway may influence the dorsal raphe by feedback from forebrain structures. It is also likely that the proposed nigroraphe projection may indeed be axonal collaterals of the nigrostriatal pathway. Which of these alternatives are relevant to the pharmacological actions of APO on the mesostriatal serotonergic system requires further investigation.

In summary, the results of the present study are consistent with the hypothesis that DA autoreceptors in the substantia nigra mediate the pharmacological actions of APO on the mesostriatal serotonergic system. The histochemical, biochemical, behavioral and histological results together strongly suggest a nigroraphe projection originating from DA cells in the pars compacta of the substantia nigra. However, possibilities of other anatomical loci and polysynaptic connections cannot be excluded as yet.

REFERENCES

1. Aghajanian, G. K. and R. Y. Wang. Habenula and other midbrain afferents demonstrated by a modified retrograde tracing technique. *Brain Res* **122:** 229–242, 1977.
2. Baring, M. D., J. R. Walters and N. Eng. Action of systemic apomorphine on dopamine cell firing after neostriatal kainic acid lesion. *Brain Res* **181:** 214–218, 1980.
3. Beckstead, R. M., V. B. Domesick and W. J. H. Nauta. Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res* **175:** 191–217, 1979.
4. Bobillier, P., S. Seguin, F. Petitjean, D. Salvert, M. Touret and M. Jouvet. The raphe nuclei of the cat brainstem: a topographical atlas of their efferent projections as revealed by autoradiography. *Brain Res* **113:** 449–486, 1976.
5. Bunney, B. S., G. K. Aghajanian and R. H. Roth. Comparison of effects of L-DOPA, amphetamine and apomorphine on firing rate of rat dopaminergic neurons. *Nature New Biol* **245:** 123–125, 1973.
6. Bunney, B. S. and G. K. Aghajanian. The precise localization of nigral afferents in the rat as determined by a retrograde tracing technique. *Brain Res* **117:** 423–435, 1976.
7. Carlsson, A. and M. Lindqvist. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacol* **20:** 140–144, 1963.
8. DiChiara, G., M. L. Porceddu, L. Vargiu, A. Argiolas and G. L. Gessa. Evidence for dopamine receptors mediating sedation in the mouse brain. *Nature* **264:** 564–567, 1976.
9. DiChiara, G., G. U. Corsini, G. P. Mereu, A. Tissari and G. L. Gessa. Self-inhibitory dopamine receptors: Their role in the biochemical and behavioral effects of low doses of apomorphine. In: *Advances in Biochemical Psychopharmacology*, vol 19, edited by P. J. Roberts, G. N. Woodruff and L. L. Iversen. New York: Raven Press, 1978, pp. 275–292.

10. Dray, A., T. J. Gonye, N. R. Oakley and T. Tanner. Evidence for the existence of a raphe projection to the substantia nigra in rat. *Brain Res* **113:** 45–57, 1976.
11. Dray, A., J. Davies, N. R. Oakley, P. Tongroach and S. Vellucci. The dorsal and medial projections to the substantia nigra in the rat: electrophysiological, biochemical and behavioral observations. *Brain Res* **151:** 431–442, 1978.
12. Fibiger, H. C. and J. J. Miller. An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat. *Neuroscience* **2:** 975–987, 1977.
13. Flicker, C. and M. A. Geyer. Behavior during hippocampal microinfusions. I. Norepinephrine and diversive exploration. *Brain Res* **4:** 79–103, 1982.
14. Geyer, M. A., W. J. Dawsey and A. J. Mandell. Fading: a new cytofluorimetric measure quantifying serotonin in the presence of catecholamines at the cellular level in brain. *J Pharmacol Exp Ther* **207:** 650–667, 1978.
15. Hjorth, S., A. Carlsson, H. Wikstrom, P. Lindberg, D. Sanchez, U. Hacksell, L. E. Arvidsson, U. Svensson and J. L. G. Nilsson. 3-PPP—A centrally acting DA-autoreceptor agonist. In: *Apomorphine and Other Dopaminomimetics*, vol 1, edited by G. L. Gessa and G. U. Corsini. New York: Raven Press, 1981.
16. König, J. R. F. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Baltimore: The Williams Wilkins Co., 1963.
17. Lee, E. H. Y. and M. A. Geyer. Selective effects of apomorphine on dorsal raphe neurons: A cytofluorimetric study. *Brain Res Bull* **9:** 719–725, 1982.
18. Lee, E. H. Y. and M. A. Geyer. Indirect effects of apomorphine on serotonergic neurons in rats. *Neuroscience* **11:** 437–442, 1984.
19. Lee, E. H. Y. and M. A. Geyer. Similarities of the effects of apomorphine and 3-PPP on serotonin neurons. *Eur J Pharmacol* **94:** 297–303, 1983.
20. Mefford, I. N. Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: Measurement of catecholamines, serotonin and metabolites in rat brain. *J Neurosci Methods* **3:** 207–224, 1981.
21. Mesulam, M.-M. The blue reaction product in horseradish peroxidase neurohistochemistry: Incubation parameters and visibility. *J Histochem Cytochem* **24:** 1273–1280, 1976.
22. Nieoullon. A., A. Cheramy, V. Leviel and J. Glowinski. Effects of the unilateral nigral application of dopaminergic drugs on the in vivo release of dopamine in the two caudate nuclei of the cat. *Eur J Pharmacol* **53:** 289–296, 1979.
23. Pasquier, D. A., T. L. Kemper, W. B. Forbes and P. J. Morgane. Dorsal raphe, substantia nigra and locus coeruleus: Interactions with each other and the neostriatum. *Brain Res Bull* **2:** 323–339, 1977.
24. Pickel, V. M., T. H. Joh and D. J. Reis. Immunocytochemical demonstration of a serotonergic innervation of catecholamine neurons in locus coeruleus and substantia nigra. *Soc Neurosci Abstr* **1:** 320, 1975.
25. Sakai, K., D. Salvert, M. Touret and M. Jouvet. Afferent connections of the nucleus raphe dorsalis in the cat as visualized by the horseradish peroxidase technique. *Brain Res* **137:** 11–35, 1977.
26. Stern, W. C., A. Johnson, J. D. Bronzino and P. J. Morgane. Influence of electrical stimulation of the substantia nigra on spontaneous activity of raphe neurons in the anesthetized rat. *Brain Res Bull* **4:** 561–565, 1979.
27. Stern, W. C., A. Johnson, J. D. Bronzino and P. J. Morgane. Neuropharmacology of the afferent projections from the lateral habenula and substantia nigra to the anterior raphe in the rat. *Neuropharmacology* **20:** 979–989, 1981.
28. Westerink, B. H. C. and J. Korf. Comparison of effects of drugs on dopamine metabolism in the substantia nigra and the corpus striatum of rat brain. *Eur J Pharmacol* **40:** 131–136, 1976.
29. Winer, B. Statistical Principles in Experimental Design. New York: McGraw-Hill, 1971.
30. Wuerthele, S. M., N. M. Friedle and K. E. Moore. Effects of intranigral administration of dopamine agonists and antagonists and baclofen on concentrations of DOPAC and dopamine in the striatum and substantia nigra of the rat. *J Neural Transm* **45:** 117–127, 1979.